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ARTICLE

Influence of exogenous salicylic acid on antioxidant enzyme activities in the roots of salt stressed tomato plants

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ABSTRACT Up-regulation of the antioxidant system provides protection against NaCl-induced oxidative damage in plants. Activities of antioxidant enzymes and hydrogen peroxide (H_2O_2) levels in the roots of tomato (*Lycopersicon esculentum* Mill. L.) plants were investigated to assess the antioxidant protection offered by long-term (3 weeks) exogenous salicylic acid (SA) pre-treatment against 100 mM NaCl induced salt stress. Our results suggest that 10^{-4} M SA was effective in increasing the activities of enzymes involved in the antioxidant system.
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KEY WORDS

Lycopersicon esculentum Mill. L.
oxidative stress
salicylic acid
salt stress

When plants are subjected to saline conditions, reactive oxygen species (ROS) like superoxide, hydrogen peroxide (H_2O_2) and hydroxyl radicals are generated (Dat et al. 2000; Farooq and Azam 2007). Salinity imposes oxidative stress on tissues that seriously disrupt normal metabolism of plants through oxidation of membrane lipids, proteins and nucleic acids in the absence of protective mechanisms (Noctor and Foyer 1998; Hernández et al. 2001). In order to avoid the harmful effects of ROS, plants evolved an effective scavenging system composed of enzymatic antioxidants, such as superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6), a H_2O_2 generating and degrading enzymes, respectively (Hajiboland and Hasami 2007).

Salicylic acid (SA) is considered as a hormone-like substance that has important role in the regulation of plant growth and development. There is strong evidence that SA mediates the oxidative burst. SA specifically binds to catalase and inhibits the activity of the enzyme (Chen et al. 1993). Thus, exogenous SA can increase H_2O_2 content of tissues, can induce the expression of antioxidant enzymes and increase plant tolerance to the abiotic stressors. In the present work salt stress acclimation of tomato plants, which had previously been grown for three weeks in low concentrations of SA (10^{-7} M and 10^{-4} M), was investigated in order to follow the changes in H_2O_2 contents of tissues.

Materials and Methods

Tomato (*Lycopersicon esculentum* Mill. cvar Rio Fuego) plants were grown hydroponically in a greenhouse under 180 µmol m^{-2} s^{-1} light intensity and at 12/12 day/night photoperiod. The temperature was maintained at 25°C and the relative humidity was 55-60%. The plants were pre-treated with 10^{-7}-10^{-4} M SA for three weeks. The salt stress was induced by 100 mM NaCl treatment for 1 week. Enzyme activities were measured after 1 week of salt stress. 750 mg of plant tissue was homogenized in 3 ml extraction buffer (50 mM phosphate buffer pH 7.0, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1% polyvinyl-polypirrolidone). After centrifugation the supernatant was used for enzyme activity assays. Superoxide dismutase (SOD) activity was determined by measuring the ability of the enzyme to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the presence of riboflavin in light (Dhindsa et al. 1981). Catalase (CAT) activity was determined by the decomposition of H_2O_2 and was measured spectrophotometrically by following the decrease in absorbance at 240 nm (Upadhyaya et al. 1985). Guaiacol peroxidase (POD) activity was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol (Upadhyaya et al. 1985). The protein contents of the extracts were determined by the method of Bradford (1976). Histochemical identification of hydrogen peroxide by 3,3'-diaminobenzidine (DAB) staining occurred according to Thordal-Christensen et al. (1997).

Results

The first line of defense against the effects of high salinity has to be induced in the root system. In order to maintain the integrity of membranes in root cells, effective enzymatic mechanisms for scavenging ROS, among them H_2O_2, were developed in plants. The activity of a H_2O_2 generating enzyme, SOD, increased after SA pre-treatments, and the activity remained much higher under salt stress than in the control plants exposed to 100 mM NaCl.

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In parallel, increased CAT activities could be detected in the roots pre-treated with 10^{-4} M SA after salt stress compared to the control. This ensured an effective decomposition of H_2O_2 in root tissues. The activity of peroxidases, which may generate or scavenge H_2O_2, decreased in the roots after salt stress.

With DAB uptake by roots, hydrogen peroxide can be localized in vivo and in situ in plant tissues (Thordal-Christensen et al. 1997). Brown marks were detectable in the root tips in control plants after 100 mM NaCl treatment. However, very few brown marks emerged within the 10^{-4} M SA pre-treated roots under salt stress, accordingly, long-term pre-treatment of tomato with both SA concentrations led to the degradation of H_2O_2 in some degree in the root tissues.

Discussion

ROS generated in cells are highly reactive in nature and destroy the normal cellular function and metabolism. Determining the role of various antioxidant enzymes in the salt tolerance of tomato, Mittova et al. (2002) found that higher salt tolerance of wild tomato (*Lycopersicon pennellii*) as compared to cultivated tomato (*Lycopersicon esculentum* Mill. L.) was correlated with increased activities of SOD and POD.

In agreement with these observations, our results also showed that oxidative stress occurred in the control plants exposed to high salinity. Our antioxidant enzyme activity measurements suggest that 10^{-4} M SA pre-treatment induced the enzymatic antioxidant system effectively by enhancing SOD and CAT enzyme activities. High activities of SOD and CAT in wild tomato (*Lycopersicon pennellii*) correlated with higher salt tolerance and better protection against oxidative stress as compared with the salt sensitive cultivated genotype (Shalata et al. 2001).

Our data suggest that 10^{-7} M SA pre-treated plants had lower capacity for scavenging ROS, generated by salt stress than 10^{-4} M SA pre-treated tomatoes. The better protection by 10^{-4} M SA results from the greater efficiency of the H_2O_2 degradation under salt stress in the leaves and roots of pre-treated plants. These mechanisms such as reduced oxidative damage have been proposed to explain the contribution of salicylic acid to the salt tolerance of tomato plants.

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